

INACTIVATION OF α - AND β -CHYMOTRYPSIN BY INTACT *HYMENOLEPIS DIMINUTA* (CESTODA)¹

PETER W. PAPPAS² AND CLARK P. READ

Department of Biology, Rice University, Houston, Texas 77001

Tapeworms, lacking a digestive tract, represent advantageous material for study of physical and chemical interactions of the host-parasite interface. Recent studies have shown that tapeworms may modify the action of host digestive enzymes, specifically those of pancreatic origin (Taylor and Thomas, 1968; Reichenbach-Klinke and Reichenbach-Klinke, 1970; Read, 1972; Pappas and Read, 1972; Ruff and Read, unpublished).

The inactivation of trypsin by the fish tapeworm *Proteocephalus longicollis* (Reichenbach-Klinke and Reichenbach-Klinke, 1970) and the rat tapeworm *Hymenolepis diminuta* (Pappas and Read, 1972) suggested that these intestinal parasites have specific protease inhibitors which protect the intact worm from host digestive enzymes. Such "anti-enzyme" protection was postulated many years ago but has largely been discredited in recent years (von Brand, 1966). The present work was undertaken to determine the possible effects of intact *H. diminuta* on α - and β -chymotrypsin.

MATERIALS AND METHODS

Specimens of *H. diminuta* from 11 day old, 30 worm infections of young male Sprague-Dawley rats (Holtzman Co.) were used in all experiments. After removal from the host, and thorough washing in 3 changes of Krebs-Ringer solution containing 25 mM Tris (hydroxymethyl) aminomethane-maleate buffer (pH 7.2) (KRT of Read, Rothman, and Simmons, 1963), worms were randomized into groups (usually 10 worms/group) and incubated in fresh KRT at 37° C for 15 min prior to their addition to the assay medium.

The assay media contained 5 ml of KRT, maintained at 37° C in a shaking water bath, plus either α - or β -chymotrypsin (45 BTEE units/mg and 28 BTEE units/mg, respectively; Sigma Chemical Co.). Groups of worms were added to the media and incubated for a predetermined time period (15 min unless otherwise noted) and removed. This period in the KRT-enzyme solution is hereafter referred to as the pre-incubation period. After removal of the worms the active enzyme was assayed by the addition of 1 ml of a prewarmed (37° C) azoalbumin (bovine origin; Sigma) solution, and the mixture incubated for 30 min at 37° C. The assay reaction was terminated by adding 1 ml of 25% (w/v) trichloroacetic acid (TCA), and the color of the TCA-soluble products measured at 420 nm following centrifugation. Some assays were also conducted using denatured bovine hemoglobin and

¹ This study was supported by a grant (AI-01384) from the National Institutes of Health, U. S. Public Health Service.

² U. S. Public Health Service Postdoctoral Fellow, 5-F02-AI-45108-02.

Hammersten casein (Sigma and Mann Research Chemicals, respectively) as substrates using the methods of Bergmeyer (1963).

Assays, using azoalbumin as a substrate, were also conducted in which worms were pre-incubated in the KRT-enzyme solution for 15 min, and not removed before the addition of substrate (*i.e.*, worms were in the assay medium for the 15 min pre-incubation and 30 min assay periods). Previous control experiments (Pappas and Read, 1972) have demonstrated that *H. diminuta* does not absorb or adsorb measurable amounts of the TCA-solution products of azoalbumin digestion.

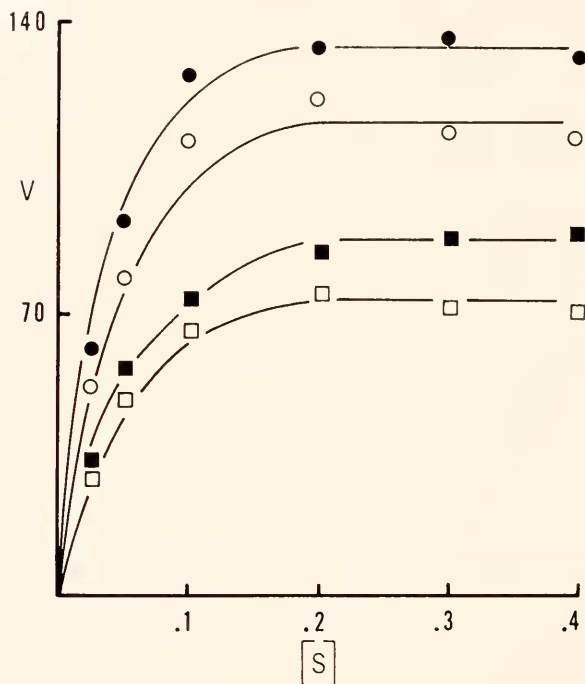


FIGURE 1. A plot of velocity (V , color change/30 min assay) of azoalbumin hydrolysis versus substrate concentration ($[S]$, % azoalbumin) by α -chymotrypsin with (open circles) and without (solid circles) a 15 min pre-incubation with *Hymenolepis diminuta* and β -chymotrypsin with (open squares) and without (solid squares) a 15 min pre-incubation with *Hymenolepis diminuta*. Lines were fitted by inspection.

Experiments were conducted in which worms were incubated in KRT for 15 min, and removed, followed by the addition of either α - or β -chymotrypsin and substrate to this same KRT. These assays demonstrated that neither enzyme was affected by excretory/secretory products of the worms. Worms have also been shown previously to lack intrinsic proteolytic activity (Pappas and Read, 1972).

Experiments were performed to determine whether *H. diminuta* inactivates α -chymotrypsinogen A (Sigma Type II, from bovine pancreas; 0.15 BTEE units/mg prior to activation, 56 BTEE units/mg following activation with trypsin). Preliminary experiments were run to insure that the α -chymotrypsinogen A was

inactive against azoalbumin, and that pre-incubation with *H. diminuta* had no activating effect on this zymogen. These experiments were conducted as above except that α -chymotrypsinogen A was substituted for the active enzyme preparation in assays with and without pre-incubation with worms. Subsequent experiments were as follows: Groups of *H. diminuta* were pre-incubated in 5 ml of KRT containing α -chymotrypsinogen A for 15 min and removed. Trypsin (Sigma Type XI; DCC treated to remove chymotryptic activity, 8000 BAEE units/mg) was then added to these and control media (no pre-incubation with worms); control and experimental assays were allowed to incubate for an additional 15 min at 37° C to allow for activation of the α -chymotrypsinogen A. Substrate was then added to all assays and proteolytic activity measured as described above. (*H. diminuta* has been shown not to excrete or secrete any anti-tryptic factors into KRT (Pappas and Read, 1972); therefore, the trypsin used as an activator would be expected to maintain full proteolytic activity.) Concurrent with the above experiments, assays were conducted to measure the proteolytic activity of the trypsin used to activate the α -chymotrypsinogen A so that this could be used as a correction factor at the end of the experiment.

TABLE I

Rates of hydrolysis of azoalbumin (0.3%), Hammersten casein (0.5%), and denatured hemoglobin (1.0%) (measured for a 30 min assay period) by α - and β -chymotrypsin with and without (control) a 15 min pre-incubation with Hymenolepis diminuta. Values listed as the mean (\pm S.E.) of 3 replicates

| | α -Chymotrypsin | | | β -Chymotrypsin | | |
|-----------------------|------------------------|--------------|--------------|-----------------------|--------------|-------------|
| | Azoalbumin | Casein | Hemoglobin | Azoalbumin | Casein | Hemoglobin |
| Control | 134 \pm 1 | 710 \pm 8* | 135 \pm 3* | 81 \pm 1 | 377 \pm 4* | 91 \pm 2* |
| Experimental | 115 \pm 2 | 632 \pm 6 | 119 \pm 2 | 64 \pm 1 | 300 \pm 4 | 76 \pm 1 |
| Per cent inactivation | 15 | 11 | 12 | 21 | 20 | 17 |

* Rates for both casein and denatured hemoglobin were determined according to the method of Bergmeyer (1963) for that of hemoglobin.

Unless otherwise noted, experiments were conducted under the following conditions: Enzyme concentration ($[E]$, α - or β -chymotrypsin) = 20 μ g/assay; substrate concentration ($[S]$, azoalbumin) = 0.3%. In addition, all points represented in the figures are the means of 3 replicates, and lines were fitted by regression analysis. The regression equations have been omitted since they are not important in interpreting the results. Without exception, the correlation coefficients of lines fitted by regression analyses were ≥ 0.99 (or ≥ -0.99).

RESULTS

Both α - and β -chymotrypsin were inactivated when pre-incubated with intact *H. diminuta*. The inactivation was not related to the substrate used in assaying chymotryptic activity (Table I). When the substrate concentration was varied at a constant enzyme concentration, both enzymes exhibited typical Michaelis-Menten kinetics (Fig. 1). Further, partially inactivated α - and β -chymotrypsin

exhibited kinetics similar to untreated enzymes (Fig. 1) indicating that the enzymes exposed to *H. diminuta* had indeed been rendered catalytically inactive rather than undergoing some kind of decreased substrate affinity. To insure that the observed inactivation was not due to the removal of some enzyme upon removal of the worms from the assay medium, assays were conducted in which the worms were allowed to remain in the assay medium for the entire assay period (45 min). With both α - and β -chymotrypsin, inactivation was greater than in assays where worms were only pre-incubated for 15 min in the presence of enzyme (Fig. 1). This indicated that the apparent inactivation was not simply due to removal of the enzyme with worms.

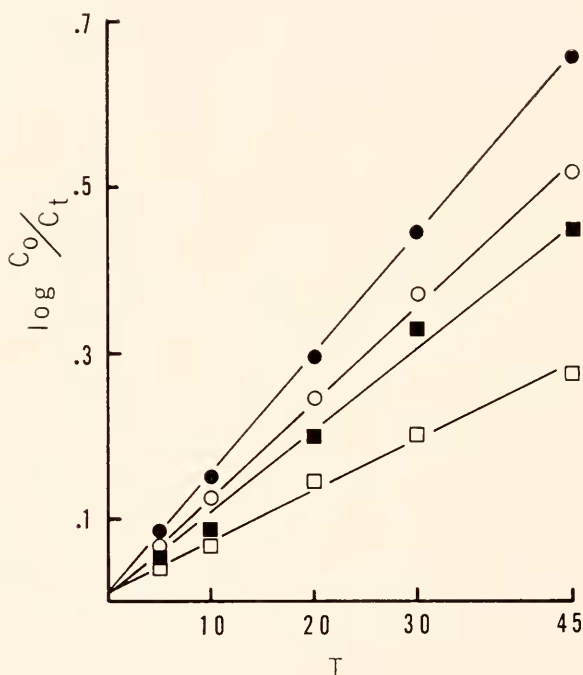


FIGURE 2. A plot of $\log (C_0/C_t)$ (where: C_0 = concentration at time 0, and C_t = concentration at time T) versus time (T , min) for the hydrolysis of azoalbumin by α - and β -chymotrypsin with and without a 15 min pre-incubation with *Hymenolepis diminuta*. All symbols as in Figure 1.

When native and partially inactivated enzymes were assayed as a function of time, hydrolysis was first order (Fig. 2); these data also showed that inactivation of both chymotrypsins was not reversible, at least within the 45 min period tested. That is, the per cent inactivation of α - and β -chymotrypsin remained constant for up to 45 min after removal of the worms.

Various concentrations of α - and β -chymotrypsin were incubated with *H. diminuta* and subsequently assayed after removal of the worms. Hydrolysis rates were not directly proportional to the enzyme concentrations (Fig. 3). This would be expected since the reactions showed first order kinetics at this substrate con-

centration (Fig. 2). The data of Figure 3 indicated that the amount of enzyme inactivated was a function of enzyme concentration only at very low concentrations; *i.e.*, at high enzyme concentrations, the absolute amount of enzyme inactivated by *H. diminuta* was constant regardless of the relative amount of enzyme present.

At enzyme concentrations of 20 $\mu\text{g}/\text{assay}$, the inactivation of α - and β -chymotrypsin was a linear function of the time of exposure of the enzymes to *H. diminuta* (Fig. 4). In this experiment worms were incubated with one of the chymotrypsins

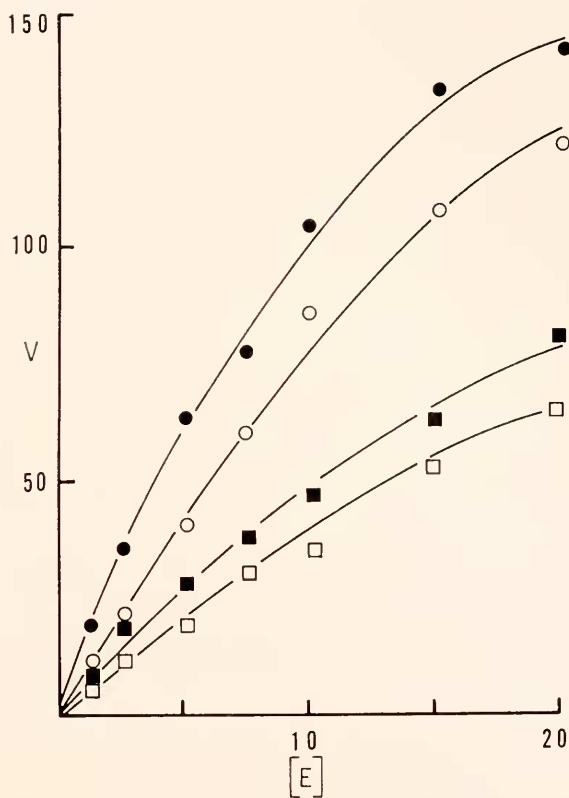


FIGURE 3. A plot of velocity (V , color change/30 min assay) of azoalbumin hydrolysis versus enzyme concentration ($[E]$, $\mu\text{g}/6 \text{ ml}$ assay) by α - and β -chymotrypsin with and without a 15 min pre-incubation with *Hymenolepis diminuta*. All symbols as in Figure 1.

for varying time periods and removed, and the medium assayed for protease activity using a fixed time period assay. At this enzyme/worm ratio, inactivation showed zero order kinetics.

When worms were incubated successively in 3 fresh solutions of α - or β -chymotrypsin, all incubations for 15 min, subsequent assays of enzyme activity revealed equal enzyme inactivation in each successive solution (Table II). Clearly, exposure to the chymotrypsins does not alter the worms' capacity to inactivate these enzymes.

TABLE II

Rates (color change/30 min assay) of azoalbumin hydrolysis by α - and β -chymotrypsin after pre-incubation with *Hymenolepis diminuta*. Worms were pre-incubated in the first set (#1) of assays for 15 min, and successively transferred to the second (#2) and third (#3) sets for 15 min each. Assays were conducted following removal of the worms. Control assays (no pre-incubation with worms) were conducted to insure the stability of the enzyme solutions.

| | α -Chymotrypsin | | β -Chymotrypsin | |
|--------|------------------------|--------------------|-----------------------|--------------------|
| | Controls | Experimentals | Controls | Experimentals |
| Set #1 | 133.50 \pm 0.96* | 105.50 \pm 1.94* | 80.66 \pm 1.91** | 64.33 \pm 1.01** |
| Set #2 | 129.75 \pm 2.01 | 106.50 \pm 1.50 | 82.33 \pm 1.09 | 66.00 \pm 1.29 |
| Set #3 | 133.00 \pm 1.29 | 108.25 \pm 1.44 | 83.00 \pm 1.36 | 65.00 \pm 1.99 |

* Values reported as mean of 4 replicates \pm S.E. Two-way analysis of variance of replicate data yielded the following: $F_{[2, 18]}$ (rows) = 1.28, $P > 0.25$; $F_{[1, 18]}$ (columns) = 392.70, $P \ll 0.001$; $F_{[2, 18]}$ (interaction) = 1.20, $P > 0.25$.

** Values reported as mean of 3 replicates \pm S.E. Two-way analysis of variance of replicate data yielded the following: $F_{[2, 12]}$ (rows) = 2.26, $P > 0.10$; $F_{[1, 12]}$ (columns) = 123.98, $P \ll 0.001$; $F_{[2, 12]}$ (interaction) = 0.06, $P > 0.75$.

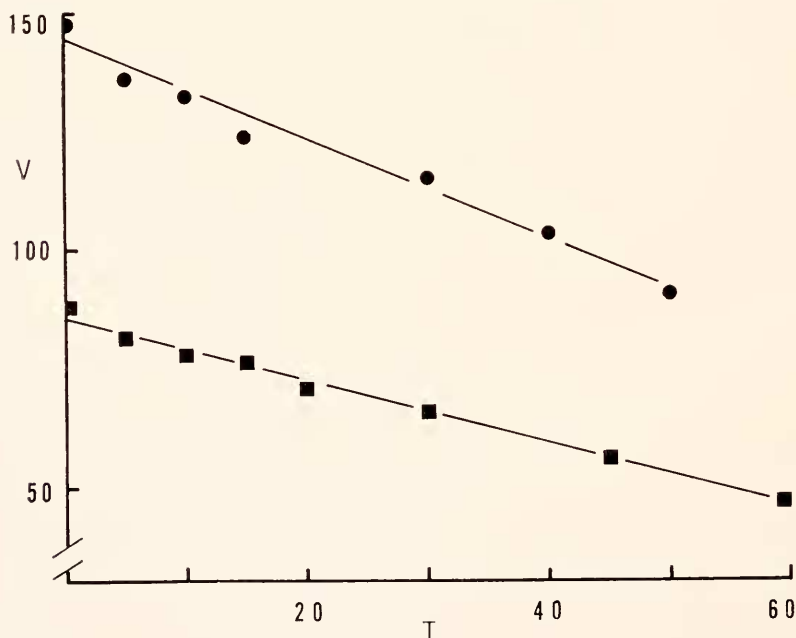


FIGURE 4. A plot of velocity (V, color change/30 min assay) of azoalbumin hydrolysis versus length of time (T, min) that *Hymenolepis diminuta* was pre-incubated in α - and β -chymotrypsin (solid circles, and solid squares, respectively).

Inactivation of α - and β -chymotrypsin was a function of the number of *H. diminuta* to which the enzymes were exposed; in the case of β -chymotrypsin, this relationship was linear up to 20 worms/assay (Fig. 5). To determine whether inactivation was a function of worm surface area rather than of worm weight, experiments were conducted using worms of identical age but varying in size (see Pappas and Read, 1972, for methods). The data (Table III) indicated that inactivation of α - and β -chymotrypsin was not a function of available surface area, but a function of total tissue weight (Fig. 4).

If surface adsorption were involved in inactivating the chymotrypsins polyions might be expected to block or interfere with inactivation. Therefore, worms were incubated with poly-L-arginine (M.W. ca. 65,000), poly-L-aspartic acid (M.W. ca. 27,000), heparin (170 units/mg) or purified yeast RNA (all at 10 μ g/ml and obtained from Sigma) for 15 min, followed by incubation with α - or β -chymo-

TABLE III

Effect of worm number, with constant worm weight, on the inactivation of α - and β -chymotrypsin by Hymenolepis diminuta. Worms were obtained, 15 days post-infection, from rats which had been infected initially with different numbers of cysticercoids (left-hand column). Values reported as the mean (\pm S.E.) of 4 replicates

| Number of cysticercoids per original infection | Number of worms/assay | Weight range** | Rate of hydrolysis* | |
|--|-----------------------|----------------|------------------------|-----------------------|
| | | | α -chymotrypsin | β -chymotrypsin |
| 10 | 2 | 909-925 | 116.50 \pm 0.50 | 87.75 \pm 1.11 |
| 30 | 6 | 878-925 | 111.25 \pm 1.44 | 92.25 \pm 1.11 |
| 50 | 9 | 907-932 | 116.00 \pm 4.06 | 93.50 \pm 2.18 |

* Measured as color change/30 min assay. Rates for control assays (no pre-incubation with worms) were as follows: α -chymotrypsin = 146.25 \pm 2.85; β -chymotrypsin = 103.75 \pm 0.48. One-way analysis of variance of replicate data yielded the following: α -chymotrypsin data, $F_{[2,9]} = 1.34$, $P > 0.25$; β -chymotrypsin data, $F_{[2,9]} = 3.80$, $P > 0.05$.

** mg wet weight of total worms in assay, listed as the weight range of the 4 assay groups.

trypsin in the presence of these same polyions. Subsequent assays, after removal of the worms, showed that inactivation of the chymotrypsins was unaffected by the addition of polyions. Control assays demonstrated that none of the above listed polyions affected either enzyme in the absence of worms.

It seemed desirable to determine whether the worms were capable of inactivating α -chymotrypsinogen A, a precursor of the chymotrypsins. Such an experiment is shown in Table IV. As indicated, worms were present only during the pre-incubation period. In C and D of Table IV, trypsin was added to activate the α -chymotrypsinogen A, and these values must therefore be corrected for this added activity by subtracting E. Thus, C minus E of Table IV gives the value for the chymotryptic activity (130 units), and D minus E gives the chymotryptic activity after exposure of the chymotrypsinogen to *H. diminuta* (81 units). Therefore, pre-incubation with *H. diminuta* resulted in a 38% decrease in the amount of α -chymotrypsinogen A activated. Appropriate controls demonstrated that worms

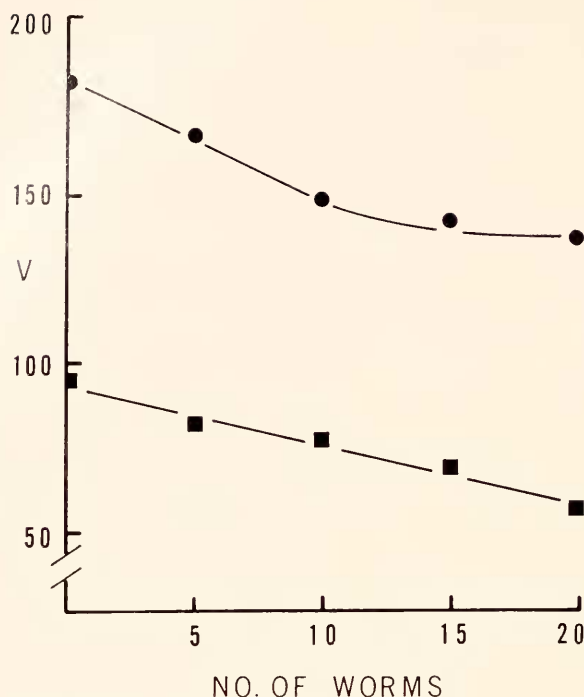


FIGURE 5. A plot of velocity (V , color change/30 min assay) of azoalbumin hydrolysis versus number of *Hymenolepis diminuta* pre-incubated with α - and β -chymotrypsin. Symbols as in Figure 4. The line for α -chymotrypsin was fitted by inspection while that for β -chymotrypsin was fitted by regression analysis.

TABLE IV

Results of an experiment to determine whether Hymenolepis diminuta inactivates α -chymotrypsinogen A. The experiment consisted of 6 different assays (A-F), each beginning with the ingredients listed in the left-hand column. After a pre-incubation of 15 min, the procedures listed in the middle column were performed, and substrate added

| Pre-incubation components | Incubation additions or deletions | Rate* |
|--|-----------------------------------|-------------------|
| (A) α -chymotrypsinogen A** | None | 1.67 ± 0.67 |
| (B) α -chymotrypsinogen A + worms | Worms removed | 0 |
| (C) α -chymotrypsinogen A | Trypsin added**† | 184.67 ± 1.67 |
| (D) α -chymotrypsinogen A + worms | Worms removed, trypsin added**† | 135.00 ± 2.65 |
| (E) Trypsin** | None | 54.67 ± 1.20 |
| (F) Trypsin + worms | Worms removed | 46.00 ± 0.57 |

* Color change/30 min assay. Means of 3 replicates \pm S.E.

** Final concentration = 20 μ g/assay.

† In these assays, the trypsin + α -chymotrypsinogen A solution was allowed to incubate for 15 min at 37° C for activation of the zymogen.

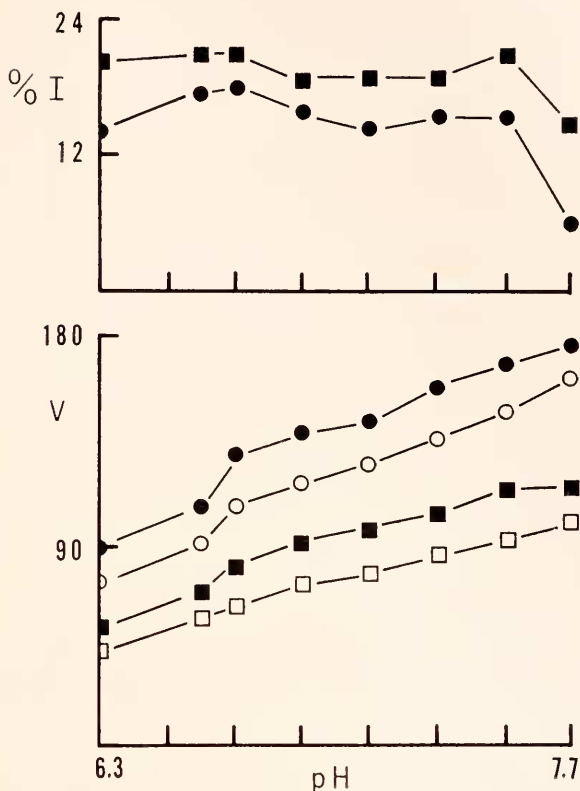


FIGURE 6. Plots of velocity (V, color change/30 min assay) of azoalbumin hydrolysis (lower graph) and per cent inactivation (%I, upper graph) versus pH of the incubation medium (0.2 pH unit increments) for α - and β -chymotrypsin with and without a 15 min pre-incubation with *Hymenolepis diminuta*. Symbols in lower and upper graphs as in Figures 1 and 4, respectively. Lines were fitted by inspection.

did not activate the inactive zymogen (A and B, Table IV). It may be noted that E minus F of Table IV shows that trypsin itself is inactivated by the intact worm as reported by Pappas and Read (1972).

When the effect of pH on the inactivation of α - and β -chymotrypsin was examined, the results were as shown in Figure 6. Between pH 6.3 and 7.5, pH clearly had a negligible effect on enzyme inactivation. There is a suggestion that there may be decreased inactivation at pH 7.7.

DISCUSSION

Some of the results of this study resemble the findings of our earlier study dealing with the effects of *H. diminuta* on pancreatic trypsin (Pappas and Read, 1972). That is, inactivation of α - and β -chymotrypsin is dependent on the period of exposure of the worms to the enzyme; inactivation appears to be irreversible; inactivation is not dependent upon the nature of the substrate nor is it blocked by polyions

which might be expected to affect electrostatic charges associated with the surface of *H. diminuta* (Lumsden, 1972). Further, inactivation does not appear to be a function of worm surface area, but rather is a function of worm weight. In all of these respects, the inactivation of the chymotrypsins resembles the effects of *H. diminuta* on trypsin.

Pappas and Read (1972) postulated that trypsin inactivation by *H. diminuta* involves the interaction of this enzyme with an inhibitor associated with the glycocalyx of this parasite. It was suggested that this inhibitor is produced continually at a rate which is a function of worm weight rather than surface area. That the glycocalyx of *H. diminuta* is continually replaced, in as short a period as 6 hr, has been shown by Oaks and Lumsden (1971), and Pappas and Read (1972) suggested that glycocalyx turnover may be more approximately a function of tissue weight than of surface area. Therefore, it is suggested that a mechanism similar to that of trypsin inactivation is also involved in the inactivation of chymotrypsins.

Simple binding of α - or β -chymotrypsin to the worm surface seems to be ruled out on several grounds: Inactivation follows zero order kinetics for periods up to 60 min; inactivation does not appear to be surface area dependent; previous exposure to the chymotrypsins does not affect the rate of chymotrypsin inactivation when the worms are exposed to fresh enzyme; and polyions are without effect on chymotrypsin inactivation.

Although the inactivation of trypsin and the chymotrypsins seem similar, there is a suggestion that the processes are separate. While the inactivation of trypsin shows a definite pH optimum at a slightly basic pH (Pappas and Read, 1972), optimal inactivation of the chymotrypsins was observed to occur over a broad pH range (6.3–7.5). This requires further study.

It is of interest that the inactivation of β -chymotrypsin is a linear function of worm number up to 20 worms/assay, whereas the inactivation of α -chymotrypsin is not. While it may be argued that this suggests separate mechanisms for the inactivation of the chymotrypsins, the pH data do not substantiate this. Further experiments will be necessary to clarify this question.

The formation of α -chymotrypsin *in vivo* involves the splitting of specific peptide and disulfide linkages of the inactive zymogen, α -chymotrypsinogen A, with the formation of π - and δ -chymotrypsin as intermediate products. In turn, β - and γ -chymotrypsin can be formed from α -chymotrypsin (Desnuelle, 1960). Since we have shown that α -chymotrypsinogen A and α - and β -chymotrypsin are inactivated in the presence of intact *H. diminuta*, we may speculate that the worm is probably capable of inactivating π -, δ -, and γ -chymotrypsin as well, since these are products of α -chymotrypsinogen A and/or α -chymotrypsin.

Although the view that parasites of the gut may utilize anti-enzymes as a mechanism for somatic protection from the digestive enzymes of the host has not been widely accepted, the present study and those of Reichenbach-Klinke and Reichenbach-Klinke (1970) and Pappas and Read (1972) suggest that the role of anti-enzymes in the ecology of intestinal parasites may require further consideration. Trypsin is inactivated by *H. diminuta*, the rat tapeworm, and tryptic activity is inhibited by intact *Proteocephalus longicollis*, a tapeworm of fishes (Reichenbach-Klinke and Reichenbach-Klinke, 1970). Although the latter authors assumed

that a trypsin inhibitor was secreted into the surrounding medium by the tapeworms, no experimental evidence for such a secretion was furnished. In the present study, and in the study of Pappas and Read (1972), it has been shown that there is no secretion of trypsin or chymotrypsin inhibitors into the medium by *H. diminuta*.

It is of interest to compare the interaction of *H. diminuta* with the chymotrypsins to the known interactions of this worm with other digestive enzymes. As noted above, inactivation of trypsin and α - and β -chymotrypsin seem to occur by similar mechanisms. However, pancreatic lipase is inhibited rather than inactivated by *H. diminuta*, and the inhibition of lipase is readily reversible (Ruff and Read, unpublished). Further, the inhibition of lipase is a function of available surface area. Pancreatic α -amylase activity is enhanced in the presence of *H. diminuta* (Read, 1972). The effects of the worm on lipase and α -amylase activity have been explained as an adsorption phenomenon, the adsorption resulting in stabilization of the enzyme in an unfavorable (lipase) or favorable (α -amylase) configuration for catalytic activity (Read, 1972; Ruff and Read, unpublished).

Membrane-bound phosphohydrolases have also been demonstrated in *H. diminuta* (Arme and Read, 1970; Dike and Read, 1971a, 1971b), but the intact worm exhibits no proteolytic activity with azoalbumin or casein (Pappas and Read, 1972), nor amylolytic activity (Read, 1972). There is also abundant evidence that the surface of *H. diminuta* is specialized for the absorption of a variety of low molecular weight metabolites. These facts, taken together, suggest that the external syncytial epithelium of *H. diminuta*, an important component of the host-parasite interface, serves a complicated digestive, absorptive, and protective function.

The technical assistance of Mr. William Kitzman is gratefully acknowledged.

SUMMARY

When specimens of intact *Hymenolepis diminuta* were incubated in the presence of α - or β -chymotrypsin, assays for proteolytic activity following removal of the worms showed an inactivation of both enzymes. The amount of inactivation in either case was dependent upon the enzyme concentration, total number of worms (total worm weight) present, period of time worms were incubated with the enzymes, and pH of the assay medium. Inactivation of α - and β -chymotrypsin was independent of available surface area and the presence of polyions, was irreversible, and ceased upon removal of the worms from the medium. Intact worms also inactivated the zymogen, α -chymotrypsinogen A. The data suggest that the inactivation of α - and β -chymotrypsin resembles that previously reported for inactivation of trypsin by intact *H. diminuta*.

LITERATURE CITED

- ARME, C., AND C. P. READ, 1970. A surface enzyme in *Hymenolepis diminuta* (Cestoda). *J. Parasitol.*, **56**: 514-516.
- BERGMEYER, H. U., Ed., 1963. *Methods of Enzymatic Analysis*. Academic Press, New York, 1064 pp.

- BRAND, T. VON, 1966. *Biochemistry of Parasites*. Academic Press, New York, 429 pp.
- DESNUELLE, P., 1960. *Chymotrypsin*. Pages 93-118 in Boyer, P. D., H. Hardy and K. Myrback, Eds., *The Enzymes*, Vol. IV. Academic Press, New York.
- DIKE, S. C., AND C. P. READ, 1971a. Tegumentary phosphohydrolases of *Hymenolepis diminuta*. *J. Parasitol.*, **57**: 81-87.
- DIKE, S. C., AND C. P. READ, 1971b. Relation of tegumentary phosphohydrolases and sugar transport in *Hymenolepis diminuta*. *J. Parasitol.*, **57**: 1251-1255.
- LUMSDEN, R. D., 1972. Cytological studies on the absorptive surfaces of cestodes. VI. Cytochemical evaluation of electrostatic charge. *J. Parasitol.*, **58**: 229-234.
- OAKS, J., AND R. D. LUMSDEN, 1971. Cytochemical studies on the absorptive surfaces of cestodes. V. Incorporation of carbohydrate containing macromolecules into tegument membranes. *J. Parasitol.*, **57**: 1256-1268.
- PAPPAS, P. W., AND C. P. READ, 1972. Trypsin inactivation by intact *Hymenolepis diminuta*. *J. Parasitol.*, **58**: 864-871.
- READ, C. P., 1972. Contact digestion in tapeworms. *J. Parasitol.*, in press.
- READ, C. P., A. H. ROTHMAN AND J. E. SIMMONS, JR., 1963. Studies on membrane transport, with special reference to host-parasite integration. *Ann. New York Acad. Sci.*, **113**: 154-205.
- REICHENBACH-KLINKE, H. -H., AND K. -E. REICHENBACH-KLINKE, 1970. Enzymuntersuchungen an Fischen II. Trypsin- und α -amylase-Inhibitoren. *Arch. Fischereiwiss.*, **21**: 72-76.
- TAYLOR, E. W., AND J. N. THOMAS, 1968. Membrane (contact) digestion in three species of tapeworm: *Hymenolepis diminuta*, *Hymenolepis microstoma*, and *Moniezia expansa*. *Parasitology*, **58**: 535-546.